REMARKS

Reconsideration is requested.

Claims 88 to 89, 101, 102, 104, 105, and 107 to 111 are pending. Claims 1-87, 90-100, 103 and 106 have been canceled, without prejudice.

The Section 103 rejection of claims 88 to 90, 101 to 102, 104 to 105 and 107 to 111 over Werness (Laboratory Investigation, Volume 76, No. 1, page 185A, March 1997, Abstract No. 1089) in view of Dunton (WO 97/16731), and the Section 103 rejection of claims 88 to 90, 101 to 102, 104 to 105 and 107 to 111 over Todorov (Laboratory Investigation, January 1998, Volume 78, No. 1, pages 73 to 78), Werness and Dunton, are traversed. Reconsideration and withdrawal of the rejections are requested in view of the following distinguishing comments.

The applicants submit that Werness and Todorov are concerned with MCM2. WO97/16731 is not concerned with MCM2, but is instead concerned with Ki-67, a different antigen which the present application demonstrates is not as useful or effective as MCM2. The presently claimed invention is specifically directed to MCM2. Knowledge of Ki-67 from WO97/16731 is submitted, with due respect, to be largely irrelevant to the presently claimed subject matter.

The purpose of the Examiner's assertion that "both references teach a nexus between Ki-67 and MB28/MCM2 in that both antigens are nuclear in location and are markers of cellular proliferation" (see, page 4 of the Office Action dated September 22, 2003 (Paper No. 14)), is not completely understood, as neither piece of information (nuclear location; marker of cellular proliferation) is believed to be sufficient to have made obvious the presently claimed subject matter.

Rather, the applicants submit, with due respect, that the Examiner has used the present disclosure, with impermissible hindsight, to piece together an assertion of obviousness by combining references that are not concerned with the same subject-matter. In particular, the teaching in WO97/16731 relating to Ki-67 provides no indication of usefulness of MCM2 in a method as presently claimed.

The Werness and Todorov references relating to MCM2 do not provide key information relating to micro-anatomical pattern of expression and discriminatory power that underlie the presently claimed invention.

The Examiner further asserts that the disclosure of Werness (although not teaching the specimens specifically recited in claim 88) "reads on the screening of a population of individuals (Claim 105)". See, page 3 of Paper No. 14. The applicants believe the Examiner's comments evidence a misunderstanding between both the technical basis for the presently claimed invention and the process steps of the claimed methods and the methods of the prior art. The Examiner appears to have overlooked the meaning of "screening" as understood by a person of ordinary skill in the relevant art. Specifically, screening will be understood by one of ordinary skill in the art to include an investigation for a rare event that may or may not occur in the tested population, i.e., (in this context) to determine whether a member of the population has a rare occurrence of dysplastic or neoplastic cells and to identify such a member from among the majority of the population that are normal. The applicants submit that performing an experiment on multiple known tumors and known normal tissues would not be considered "screening of a population".

More specifically, in a medical context, "screening" is defined by the United Kingdom National Screening Committee as

> "The systematic application of a test or inquiry, to identify individuals at sufficient risk of a specific disorder to warrant further investigation or direct preventive action, among persons who have not sought medical attention on account of symptoms of that disorder." See, Muir Gray JA "Screening", Chapter 3.6, pp68-73 in : Oxford Textbook of Medicine, 4th edition, vol 1, 2003, Eds: Warrell et al.

A person of ordinary skill working in the cancer screening field would not regard testing tissue samples known to be tumor or normal as equivalent to or encompassed within the meaning of "screening a population of individuals".

In relation to Werness, the Examiner states that one of ordinary skill in the art would have allegedly been motivated to make the presently claimed invention from the cited art

> "because Werness *et al.* successfully demonstrated that BM28/MCM2 is a diagnostic marker that is preferentially expressed in neoplastic cells versus normal proliferating cells. Extrapolating this knowledge to tumor cells derived from fluid samples is a mere variation from histological analysis of frozen tumor tissue cells as exemplified in the teaching of WO97/16731". See, page 4 of Paper No. 14.

The applicants respectfully submit however that Werness could not have provided such motivation as both the extrapolation and the characterization to WO97/16731, which provides no teaching relating to MCM2, is incorrect. That is, combination of Werness and WO97/16731 would not have been expected by one of ordinary skill in the art, absent an impermissible use of hindsight, as the documents are

concerned with different proteins, i.e., Werness employed BM28 or Mcm2 while Sunton et al used Ki67.

As noted above, the Examiner's assertion that Mcm2 and Ki67 have a "nexus" between them ", in that both antigens are nuclear in location and are markers of cellular proliferation, is not believed to be sufficient to allow any conclusion that either or both could be used in the context of the presently claimed invention.

The Examiner states, with reference to Werness,

> "with 70/72 frozen tumor samples (97%) positively expressing BM28/MCM2, one of ordinary skill in the art would have a reasonable expectation of success that cellular samples [as defined in the claim] would also be diagnostic of dysplasia or neoplasia". Id.

The Examiner has not shown that there is any indication in the cited art however that it would have been obvious to have used MCM2, or even obvious to have tried to use MCM2 (even though the latter is not sufficient to establish a *prima facie* case of obviousness), in the context of the subject-matter of the present claims. The Examiner's statements and basis for the rejections rely on an impermissible use of hindsight.

The Examiner is urged to appreciate that screening for cellular growth abnormalities typically involves detection of abnormal cells (if present) in cytological specimens. The cells that are sampled in such specimens are generally derived from the surface of an epithelium covering or lining an organ. Cells from certain organs can be sampled actively (for example by scraping or brushing) but for many organs they are sampled passively. That is, the cells available for examination are those exfoliated or sloughed spontaneously into a bodily fluid.

The Examiner is believed to have incorrectly characterized Werness et al. as successfully demonstrating diagnosis or teaching that Mcm2 is "diagnostic of dysplasia or neoplasia".

A distinction which is important in the screening context between the teaching of Werness et al. and Todorov et al. and the presently claimed invention. Specifically, when looking at samples of unknown condition in order to determine whether or not they are dysplastic or neoplastic, one does not know in advance whether the samples are dysplastic or neoplastic. In Werness et al. and Todorov et al. all samples were known to be tumor or normal. Using Western blotting, the authors showed that 70/72 tumors contained detectable Mcm2, but also that many normal tissues contained detectable Mcm2: 18% in Werness et al.; 27% in Todorov et al.. Furthermore, Werness et al. state that in tissue sections there were "strong nuclear signals in normal proliferating cells". Todorov et al. provide quantitative immunohistochemical data for 12 cases of intraductal and invasive breast carcinoma, in which the mean number of Mcm2-positive nuclei was approximately 50% (range 10->80%). Werness et al. and Todorov et al. did not consider the distribution of Mcm2 expression within normal tissues and within tumors, and did not suggest that in tissues expressing Mcm2 there is a difference in Mcm2 distribution between normal tissue and tumor tissue. From their experiments it is impossible to conclude or have any reasonable expectation that positional information relating to differences in distributions of Mcm2 expression could be used to discriminate between normal tissues and tumors in screening of cytological specimens from a range of anatomical sites. Mcm2 is found in both tumors and normal tissue, as Werness et al. and Todorov et al. showed. Thus, the mere presence of Mcm2 in tissue, as identified

for example by performing Western blotting as in Werness et al. and Todorov et al., is not diagnostic of dysplasia or neoplasia. Normal tissue has Mcm2 as well. The Examiner does not explain how an ordinarily skilled person performing a Western blot experiment as taught by Werness et al. on a sample for which it is not known whether the tissue is normal or tumor could have made any diagnostic conclusion if he found Mcm2. To reiterate, Werness et al. and Todorov et al. demonstrate that Mcm2 is found in both tumor and normal tissue.

Critical experimental results underlying the presently claimed invention include the applicants demonstration of the difference in the distribution of expression of Mcm2 in normal tissue and tumors. The applicants have demonstrated that Mcm2 persists to the surface of dysplastic and neoplastic tissue, whereas it is restricted to basal and suprabasal layers of normal tissue. It is this novel observation that allows Mcm2 to be used for screening cytology. Only with this information was it possible to know that one can discriminate between normal and tumor tissues by testing samples as defined in the present claims. The results of the applicants experiments were not predictable or obvious from Werness et al. nor Todorov et al., either alone or together, or with the Examiner's further cited reference. These documents do not teach or suggest testing of cytology samples as defined in the claims. Furthermore, there would have been no motivation to test such samples since based on information from these documents there was no reasonable expectation of any discriminatory or diagnostic benefit in doing so.

Furthermore, the results were not predictable from Dunton et al. as the authors there were concerned with a different antigen, Ki-67, the properties and usefulness of which were not predictive for Mcm2.

Werness et al. (and Todorov et al. Laboratory Investigation, January 1998, Vol. 78, No. 1, pages 73-78, discussed further below) concerns work using tissue sections only and makes no mention whatever of screening using cytological samples, as presently claimed. These are very different settings: it is not the case that analysis of cytology samples is "a mere variation from histological analysis", as asserted in the sentence spanning pages 5 and 6 of Paper No. 14.

Werness et al. (and Todorov et al.) include no comment, teaching or suggestion as to the expression of Mcm2 in surface epithelial layers. This is the basis on which differences in the amount or pattern of Mcm2 expression in cytology samples enable screening of a population of individuals for cellular growth abnormalities. There were therefore no grounds for a reasonable expectation that Mcm2 would be effective in the screening setting.

There is nothing in Werness or Todorov providing any positional information for MCM2 expression in tumors. Only tissue sections and Western blots (carried out on solubilized protein lysates, with no remaining cellular or tissue structure). It would not have been obvious from either of these references that MCM2 is expressed at the surface of any tumors, nor that it will provide for a discriminatory screen to test for evidence of tumors among samples when it is not known whether or not there are any tumors present and if so in which samples. The experimental evidence in the present application includes demonstration of full-thickness staining for MCM2 in tumors and in premalignancies (but not normal), a result that contrasts with what is seen for Ki-67 (subject of WO97/16731), and is not predictable from the cited art. See, for example, page 21, line 13-page 14 line 10, and page 31, lines 9-24, page 22, line 19, of the

present specification and various experiments showing full thickness staining and discussion of analysis of cervical smears that sample only the tissue surface, e.g. page 41, lines 1-13, also page 55, lines 1-15, and pages 64-66 for analysis of urine cytology smears, and pages 67-68 for analysis of fecal smears, also mentioned at page 73, lines 9-19.

Again, the Examiner is urged to appreciate that Western blotting, as performed by Werness et al. and Todorov et al., uses lysed samples and enables no conclusions to be drawn regarding the micro-anatomical distribution of Mcm2 in epithelial layers. Werness et al. states that immunohistochemistry of tissue sections showed staining of "most nuclei" of tumors for Mcm2. The distribution of these positive cells is not commented on in Werness et al. (nor Todorov et al.). There was no comment, suggestion or teaching in either Werness et al. or Todorov et al. as to whether or not the surface layers of cancers were positive for Mcm2.

The Examiner asserts, in dismissing the applicants previous comments and as a basis for maintaining the obviousness rejection, that the cited documents teach that "cells can be taken from epithelial tumors and analyzed for their expression of BM28/Mcm2". See, page 6 of Paper No. 14. Even if true, the applicants respectfully submit that this would not have been adequate to identify cases of malignancy usefully. That is, there would be a high probability that normal tissues would also test positively in this setting, as most cases of normal tissue also contain Mcm2 positive cells, as taught by Werness et al.: "Tissue sections reacted with anti-BM28 gave strong nuclear signals in normal proliferating cells". The ability of a marker to discriminate between normal tissue and cancer or pre-cancer in cytology specimens is due to the difference in

expression in the surface layers of normal tissue compared to those of cancer/pre-cancer. The differences in expression of Mcm2 that the applicants observed in the surface layers of normal tissue and cancer/pre-cancer were not obvious from Werness et al. and/or Todorov et al. alone, in combination, or in combination with WO97/16731, as described below.

The applicants believe that a scientist performing an experiment in accordance with Werness and finding MCM2 would not have been able to predict from that finding whether the tissue in the tested sample was normal or tumor. The applicants believe that Werness et al. indicates that 18% of normal tissue tested in their experiments tested positive for MCM2.

Werness et al. and Todorov et al. did not provide any reasonable expectation that Mcm2 would prove successful at identifying in cytology specimens cells released from the surface of cancers or pre-cancers and therefore be a valuable marker for screening of populations for cellular growth abnormalities using cytology samples. Werness et al. and Todorov et al. did not therefore teach or suggest a method for determining the presence or absence of dysplastic or malignant cells in a test, or cytology, sample containing cells from an individual, as claimed. The presently claimed invention is based on the unexpected finding that there is full thickness expression (including abundant expression in surface layers) of Mcm2 in cancer and pre-cancer affecting a wide variety of anatomical sites.

As for Dunton et al.'s work relating to Ki67, the applicants submit the same cannot and does not provide any suggestion, alone or combined with the other cited art, of the claimed invention. Specifically, for example, Dunton et al., does not provide any

indication that Mcm2 could be employed in the presently claimed invention. Furthermore, the applicants work demonstrates that Ki67 is not useful in the context of the claimed invention, whereas, surprisingly, Mcm2 is. The applicants observed a very high frequency of expression of Mcm2 in the surface layers of precancerous and/or cancerous cervical and bladder epithelium. Frequency of expression of Ki67 in these surface layers was much less. There was a much higher frequency of staining for Mcm2 compared with Ki67 in tumor cells from breast and colon, and Mcm2 has been shown to be of value as a marker for early detection, using cytological samples, of a variety of common cancers and precancers, including those of large bowel, larynx and oesophagus.

WO97/16731 (Dunton et al) is concerned with the cervical smear test. Ki67 was considered as a marker for identifying individuals with cervical squamous intraepithelial lesions precisely because it "is expressed in upper epithelial levels of intraepithelial neoplasia of the cervix" (page 12 of WO97/16731). In WO97/16731 there was a correlation between histological data and cytological findings for cervical smears, where cells are actively sampled from the cervix by scraping or brushing.

However it was not possible to extrapolate generally from WO97/16731 (which applies only to active sampling of Ki67-positive cells from the cervix) that markers expressed in sections of tissue from other sites will inevitably also be found in cytological specimens, including those sampled passively. To say that examination of cytological samples is "a mere variation from histological analysis of frozen tumor tissue" is, with due respect, submitted to be grossly over-simplified and factually incorrect.

The teachings of WO97/16731, when combined with those of Werness et al. and/or Todorov et al., individually or in combination, would not have provided a reasonable expectation that Mcm2 would make it possible to provide a high throughput screening of cytological specimens from a range of anatomical sites for cellular growth abnormalities.

On examination of pre-malignant conditions the inventors showed very frequent expression of target polypeptides, including in surface cells. It is most desirable to detect neoplasia in the pre-malignant stage, when the disease is not life threatening. Examples of such conditions include cervical squamous intraepithelial lesions; non-invasive bladder carcinoma; colorectal adenomas and dysplasia/intraepithelial neoplasia of the mucosa of the lung and upper aero-digestive tract.

Moreover, pre-malignant disorders are biologically different to the malignant conditions examined by the cited art. It was not obvious from the cited art that the target polypeptides would be expressed in abundance in the surface layers of pre-malignant disorders and could serve as biomarkers for the detection of such disease. It is also very different to provide a clinically useful diagnostic test to determine, potentially on a large, high through-put scale, whether or not individuals have a malignant or pre-malignant disorder, in contrast to the analysis of the cited art of known tumor samples compared with known normal samples.

Since, according to Werness and Todorov, both normal and tumor tissues can apparently test positive for MCM2 (they report 18% of normal tissues tested), finding MCM2 by performing Western blotting is not diagnostic of dysplasia or neoplasia. An ordinarily skilled person performing an experiment as taught by Werness on a sample

for which it is not known whether the tissue is normal or tumor could not make any diagnostic conclusion if MCM2 were found. Werness demonstrates that MCM2 is found in both tumor and normal tissue.

On the contrary, the presently claimed invention allows for discrimination between normal samples and those with dysplasia or neoplasia (when the state of the tissue tested is not known in advance), based on the unexpected experimental findings as set out in the patent application.

The applicants believe that testing known tumor samples in tissue section or solubilized extract and known normal samples to show a difference in staining for a target antigen as between the tumor and normal is quite a different thing to realize and demonstrate that there is an abnormal abundance and pattern of target expression that allows for clinical sampling and testing of material from individuals for whom it is not known whether or not they have any cellular growth abnormality. In running a clinical or diagnostic test on an individual and especially when screening a population of individuals, such as is done on a massive scale for example for cervical cellular growth abnormality, it is important to be able to take a sample from the individual easily and quickly and to have a test that is accurate, sensitive and subject to rapid through-put. Such is provided by the presently claimed invention and would not have been obvious from the cited art.

Withdrawal of the Section 103 rejections and a Notice of Allowance are requested. The Examiner is requested to contact the undersigned in the event anything further is required in this regard.

Respectfully submitted,

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